REVIEW

Immunologically-mediated tumour cell apoptosis: the role of TRAIL in T cell and cytokine-mediated responses to melanoma

Tam Nguyen, Wayne Thomas, Xu Dong Zhang, Christian Gray and Peter Hersey

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Immune responses against human melanoma are common and are believed to influence the natural history of the disease. In particular, CD4 T cell infiltrates are associated with regression of primary melanoma and with responses to treatment with interferon-α2 (IFN-α2). Our studies have shown that CD4 T cells appear to kill melanoma by means of a member of the tumour necrosis factor (TNF) family expressed on their surface and called TNF related apoptosis inducing ligand (TRAIL). Moreover, sensitivity to TRAIL also predicts responsiveness of melanoma to CD4 T cells. TRAIL is not expressed on resting lymphocytes but is expressed at high levels after exposure to IFN-α2 and on activated T cells. Lymphocytes from melanoma patients in early stages of the disease show high levels of expression after exposure to IFN-α2 and IFN-γ but expression was less on lymphocytes from stage IV patients. This may be due to factors from melanoma cells in that supernatants from some melanoma cultures suppressed IFN-α2 upregulation of TRAIL. Sensitivity of melanoma cells to TRAIL can be increased by inhibition of the activation of NF-κB and anti-apoptotic events downstream of NF-κB. These results suggest that TRAIL may be an important mediator of responses against melanoma induced by immunotherapy or by treatment with IFN-α2 and interleukin-2. Studies on surgical biopsies of melanoma however show that fresh isolates appear less sensitive to TRAIL-induced apoptosis and effective therapy may involve combinations with other agents.

FORUM Trends in Experimental and Clinical Medicine, 10: 243-252, 2000

Key words: TRAIL, apoptosis, IFN-α, IFN-γ, CD4 T cells, melanoma

Introduction

Melanoma has long been regarded as a model human cancer to study immune responses against tumours. Many primary melanomas have areas of regression associated with lymphoid infiltrates (1) and lymphoid infiltrates were shown to be a favourable prognostic indicator in long-term follow up studies (2). Primary melanoma was associated with infiltration by CD4 T cells (3, 4) and these cells predominate in regressing lesions (5). In contrast CD8 T cells predominated in metastases (3). Infiltration of metastases with CD4 T cells during treatment with IFN-α2 was also shown to correlate with clinical responses (6).

The mechanisms by which CD4 T cells could mediate regression has attracted much inte-

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Abbreviations: TRAIL, TNF-related apoptosis inducing ligand; FLIP, FLICE (caspase 8) inhibitory protein; CD40L, CD40 ligand; FasL, Fas ligand; AJCC, American Joint Committee on Cancer; Ca, calcium; Cr, chromium; sn., supernatant; TRAIL-R, TRAIL-receptor; NF-κB, nuclear factor kappa B; Bcl-2, B cell lymphoma gene 2; IAP, inhibitors of apoptosis proteins; XIAP, X-linked inhibitor of apoptosis

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rest as their content of cytotoxic granules is much lower than that in NK cells and CD8 T cells. In view of this, much attention has been given to their role in killing target cells by an alternative killing mechanism that depends on interaction of ligands of the TNF family with receptors on the target cell, viz. Fas, TNF-α receptors or receptors for TRAIL. Some studies have suggested that CD4 T cells kill target cells solely by the latter mechanism (7). Both granule exocytosis and TNF family receptor/ligand dependent mechanisms induce apoptosis in the target cells by activation of caspases. In the case of granule-dependent killing, granzymes released from the granules appear to activate procaspases whereas the TNF receptor family interactions depend principally on activation of caspase 8 at the cell membrane and subsequently effector caspases such as 3, 6 and 7 (8). Granule dependent mechanisms may also involve as yet poorly defined caspase-independent mechanisms of apoptosis induction.

Studies on perforin-deficient mice suggested that the granule exocytosis pathway may be the principal mechanism involved in rejection of tumours (9) but several recent studies suggest that the cell membrane signalling pathways may be more important in tumour rejection than suggested by the studies on perforin-deficient mice. Transmembrane signalling can be inhibited by overexpression of FLIP but does not however inhibit the granule exocytosis pathway. In studies by Medema et al. (10) it was shown that injection of tumour cells transfected with FLIP resulted in tumour development whereas tumour cells with low levels of FLIP were rejected. Furthermore, tumours escaping from rejection had high levels of FLIP. Similar findings were reported in studies on another tumour model by Djerbi et al. (11). These studies therefore suggest that the transmembrane signalling pathway may be more important in tumour rejection than at first thought.

TRAIL appears to be responsible for killing of melanoma cells by CD4 T cells

In view of the possible involvement of the TNF-family receptors in the killing of target cells by CD4 T cells, a study of the role of TNF ligands known to be involved in the induction of apoptosis was carried out. These studies showed that TNF-α, CD40L and FasL were not involved in killing of melanoma but inhibition of TRAIL with moAb showed that practically all the killing of melanoma by CD4 T cells was due to their expression of TRAIL. In contrast, killing of melanoma cells by CD8 CTL appeared to be largely independent of TRAIL (12).

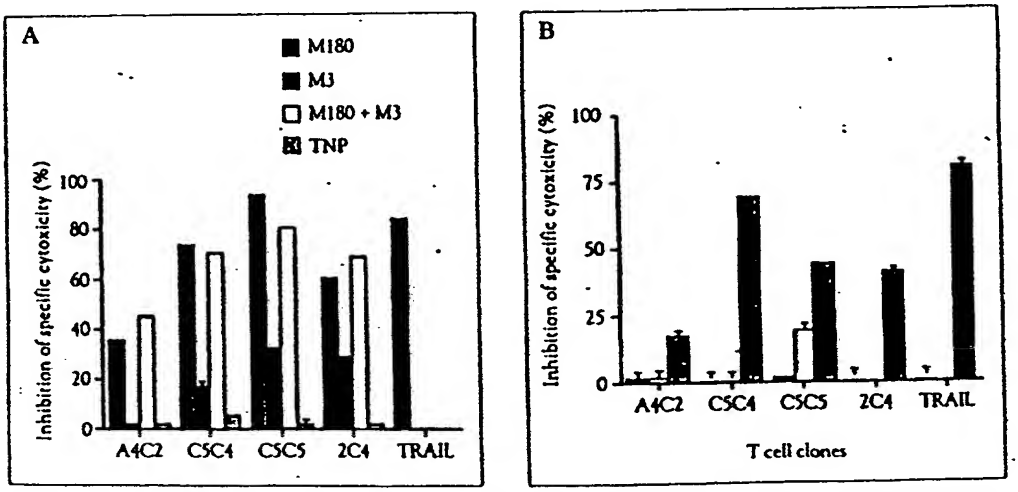

Figure 1. Inhibition of CD4 T cell mediated killing of; A) Jurkat T cells and B) allogeneic melanoma cells, Mel-CV by moAb (M180) which specifically block TRAIL activity. A4C2, C5C4, C5C5 and 2C4 are T cell clones generated from a melanoma patient. Reproduced with permission from (12)

Importantly, we also found that susceptibility of melanoma lines to killing by a panel of CD4 T cell clones correlated with their susceptibility to killing by TRAIL. The exception was killing of the K562 myeloid target cells, which were not killed by the CD4 T cell clones but were killed by TRAIL. We surmise that this result indicates that specific antigen recognition is needed to induce TRAIL expression. The correlation between susceptibility of melanoma cells to killing by CD4 T cells and apoptosis induced by TRAIL suggests that susceptibility of melanoma to immunotherapy may be determined largely by their susceptibility to apoptosis induced by TRAIL as well as expression of antigens recognised by T cells.

TRAIL is induced on blood lymphocytes from normal subjects and melanoma patients by IFN-α and IFN-γ

Studies by Kayagaki et al. (13) drew attention to an important role for IFN-α in the upregulation of TRAIL expression on T cells stimulated by anti-CD3 moAb. Peak expression of TRAIL was shown at six hours on both CD4 and CD8 T cells. IFN-γ, IL-2 and a variety of other cytokines did not upregulate TRAIL under the same conditions. Griffith et al. (14) showed that TRAIL was upregulated on human monocytes by both IFN-α2 and IFN-γ, and that the death receptor TRAIL-R2 was downregulated. Similarly, immature human NK cells were shown to express TRAIL and to mediate apoptosis *via* TRAIL expression (15) IL-2 appeared to be an important regulator of TRAIL expression on human NK cells (16).

In view of these findings we have investigated the role of IFN-α and IFN-γ in expression of TRAIL on blood lymphocytes from patients with melanoma using flow cytometry to gate on lymphocyte subpopulations and sensitive avidin-biotin detection methods to detect TRAIL. The results are summarised in Figure 2 and indicate several findings. Firstly, unstimulated PBL from most normal subjects did not express TRAIL but when PBL were exposed to IFN-α (200 IU) for 16 hours there was a substantial increase in TRAIL expression, particularly on CD4 T cells. TRAIL was also upregulated on CD8 T cells and CD14 positive monocytes.

Secondly, studies on melanoma patients showed that there was substantial expression of TRAIL on "resting" lymphocytes in the absence of IFN-α or IFN-γ. This was evident even in patients with primary melanoma at long intervals after removal of the primary melanoma (AJCC stage I and II melanoma). Similar levels of expression were found on PBL from patients with a history of regional lymph node metastases (AJCC stage III melanoma). Exposure to IFN-α induced sub-

Table 1. Cytotoxic activity of CD4 T cells against autologous and allogeneic melanoma target cells compared to TRAIL-induced apoptosis. Reproduced with permission from (12)

Cell Line	% TRAIL-induced apoptosis	Specific cytotoxicity*			
		K562	C5C4	C5C5	2
ME4405	61	35	58	17	7
Mel-CV	54	47	62	26	45
Mel-RM	76	92	83	32	64
Mel-FH	24	80	36	54	80
ME1007	0	3	3	0	1
Mel-JS	0	3	3	0	2
K562	26	3	1	0	0

* *Values >6 were significantly (±2 SD) above baseline spontaneous release*

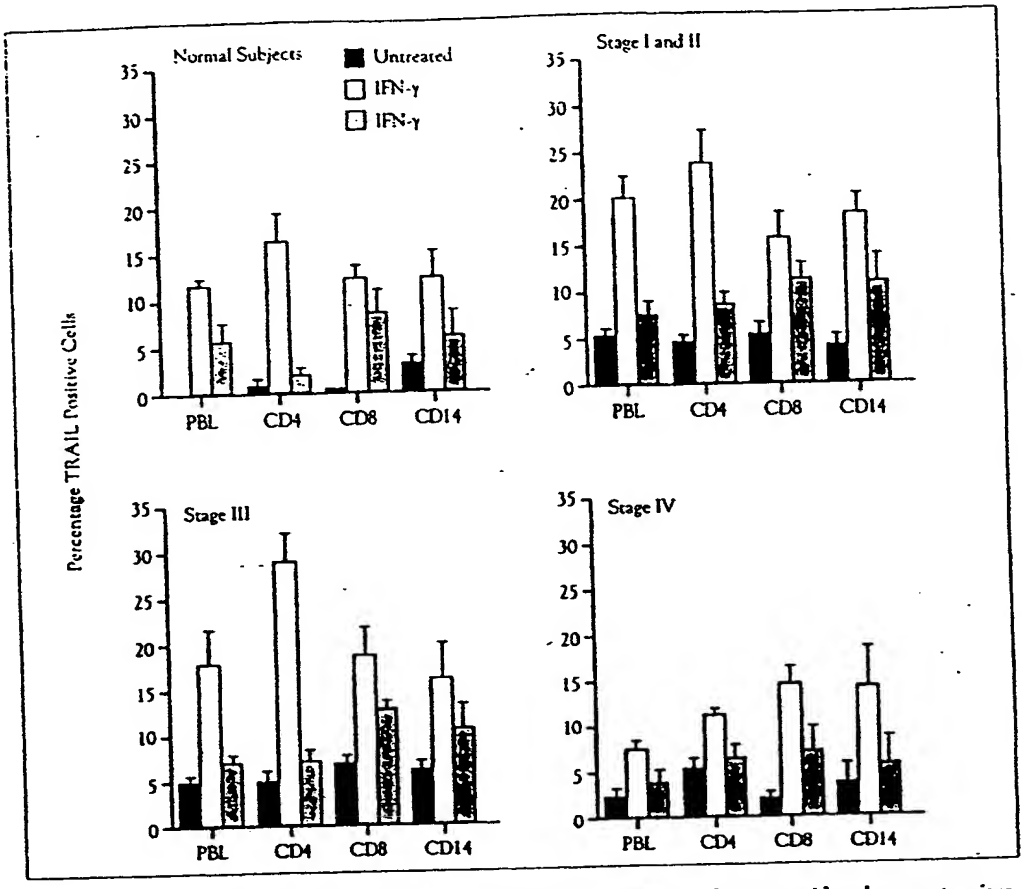

Figure 2. TRAIL expression on blood lymphocytes taken from normal subjects and patients with melanoma at various stages of their disease. CD4, CD8 and CD14 (monocyte) populations were identified by gating on these populations with moAb against the sub-populations. TRAIL expression was identified with the M181 (10 μg/ml) on ice for 1h followed by biotinylated rabbit $F(ab)_2$ anti-mouse Ig and rhodamine phycoerythrin conjugated streptavidin. (Methods are also described in Current Protocols in Immunology, Vol. 1, Chapters 4, 5, 10) Numbers of normal subjects, stage I and II, III and IV pts were 10, 16, 10 and 9 respectively

stantial increases in TRAIL expression again predominantly on the CD4 subset but also on CD8-positive subsets. Expression was less marked on PBL from patients with disseminated melanoma (AJCC stage IV melanoma) and addition of IFN-α to the CD4 T cells induced significantly lower increases in TRAIL expression than in patients with earlier stages of melanoma. None of the patients with stage IV melanoma had received prior chemotherapy.

Thirdly, addition of IFN-γ at 200 μl/ml for 16 hours also increased TRAIL expression. This was not as marked as that seen with IFN-α2 and was more marked on CD8 T cells and monocytes compared to that on CD4 T cells.

LAK cells express high levels of TRAIL and mediate TRAIL-dependent killing of target cells in Ca^{2+} free media

To examine whether TRAIL may mediate killing of target cells by LAK cells we generated LAK cells by culture of PBL in IL-2 (500 μl) for two weeks. This resulted in a population enriched in CD3-negative CD8-positive NK cells and high TRAIL expression (Figure 3). IFN-γ did not increase TRAIL expression on the LAK cells.

LAK cells have high levels of cytotoxic granules and are believed to mediate their killing by perforin granzyme release. We examined

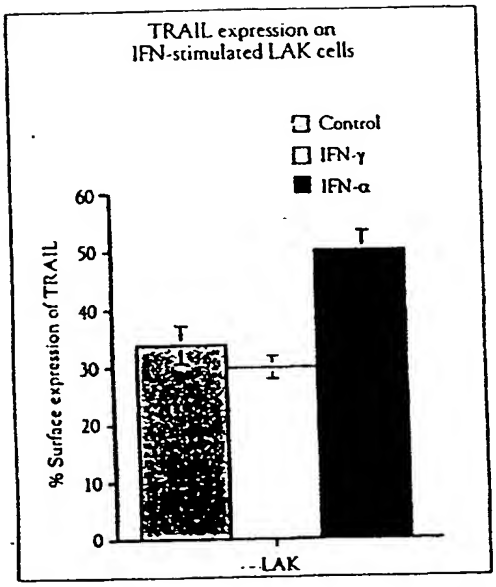

Figure 3. TRAIL expression of LAK cells. PBL grown in 500 μl of IL-2 for 2 wk. Cells were over 90% CD3-negative CD56-positive

the role of TRAIL in killing by LAK cells by assay in Ca^{2+} free media as it is known that killing by FasL, TRAIL and TNF-α is calcium independent, whereas the granular exocytosis mechanism is calcium dependent. The results shown in Figure 4 are representative of two experiments and indicate that killing by IFN-α2 stimulated LAK cells is mediated in part by TRAIL. This is shown by lysis of MM200 and IgR3 melanoma cells in Ca^{2+} free media (30-50% lysis) and inhibition of this killing (in Ca^{2+} free media) in part by moAb to TRAIL. (The remainder of the killing may be mediated by other ligands such as TNF-α and Fas-L but this was not examined.)

Supernatants from some cultures of melanoma cells inhibit IFN-α upregulation of TRAIL expression

Studies on IFN-α2 upregulation of TRAIL on lymphocytes from patients with stage IV disseminated melanoma showed that expression of TRAIL was less than on PBL from patients with earlier stages of melanoma. In certain patients no increase in TRAIL expression was detected at all. We questioned whether this may be due to tumour-derived factors by testing the effect of supernatants from melanoma cultures on IFN-α induced upregulation of TRAIL. As shown in Figure 5, supernatants from two cultures resulted in

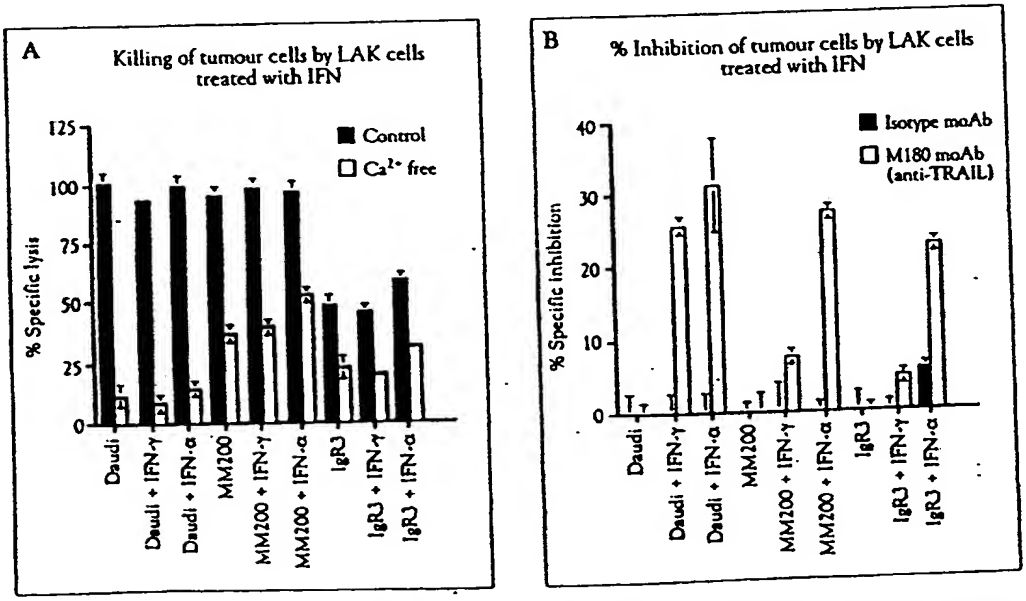

Figure 4. A) Involvement of TRAIL in killing by LAK cells. LAK cells produced as described in the text and stimulated with IFN were tested for their cytotoxic activity in ^{51}Cr release assays in media containing Ca^{2+} or Ca^{2+} free media. Significant lysis of melanoma but not Daudi cells was seen in Ca^{2+} free media. B) This was due in part to TRAIL, as shown by inhibition of CTL activity by the moAb against TRAIL, M180

complete inhibition of IFN-α2 induced upregulation of TRAIL expression. The nature of the factors inhibiting upregulation of TRAIL is the subject of further study.

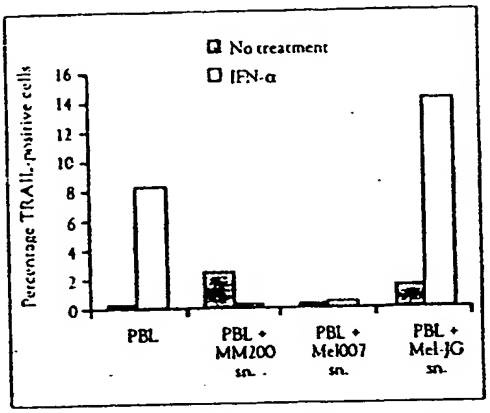

Figure 5. Inhibition of IFN-α2 induced upregulation of TRAIL expression by sn. from the melanoma lines MM200 and Me1007. sn. from the Mel-JG line were non-inhibitory. (sn. collected from 2 million cells in 1ml of AIM V media for 24 hours. sn. were added at the same time as IFN-α2)

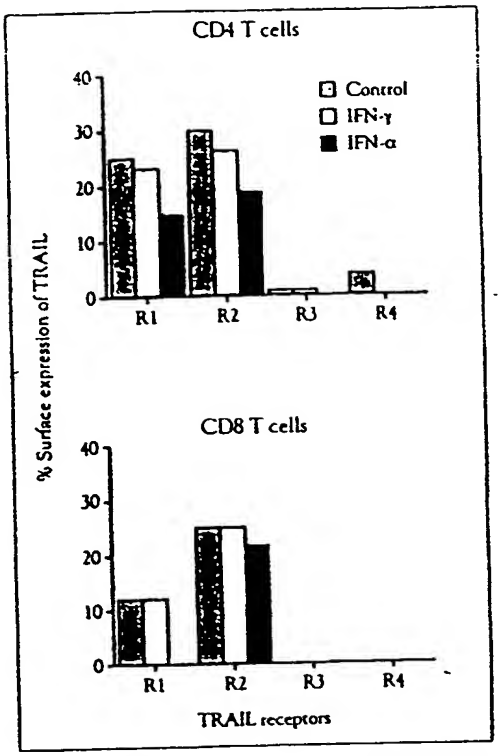

Figure 6. TRAIL receptor expression on CD4 and CD8 T cells were isolated on MACS CD4⁺ and CD8⁺ T cell isolation kits respectively

Resting lymphocytes express low levels of death receptors, TRAIL-R1 and R2, and LAK cells have no detectable surface expression of TRAIL-R

Previous studies on melanoma cells have shown that their susceptibility to TRAIL induced apoptosis was determined largely by the level of expression of the death receptors on their surface (17, 18). IFN-γ had no effects on TRAIL-R expression on melanoma. IFN-α also had minimal effects on TRAIL-R expression in general but did result in small decreases in some melanomas.

To study receptor expression on lymphocytes, resting PBL were separated on MAC columns into CD4 and CD8 T cells and exposed to IFN-α or γ. As shown in Figure 6, both CD4 and CD8 T cells expressed low levels of TRAIL-R1 and R2. IFN-γ had no effect on receptor expression but IFN-α caused down-regulation of both receptors on CD4 T cells and TRAIL-R1 on CD8 T cells. LAK cells had no detectable expression of TRAIL-R but studies on permeabilised cells showed that low levels of TRAIL-R2 and the decoy receptor TRAIL-R3 were present. These results suggest that lymphocytes are not protected by expression of decoy receptors but instead are most likely protected from TRAIL induced apoptosis by low expression of the death receptors.

Melanoma cells from fresh isolates appears relatively resistant to TRAIL-induced apoptosis

The results described above suggest that TRAIL may be an effective agent in the treatment of melanoma. However, studies on melanoma freshly isolated from surgical biopsies show a disturbing degree of resistance to TRAIL. This appears to be associated with low expression of the death receptors for TRAIL on the melanoma cells. Once established in culture there is an increase in TRAIL death receptor expression and sensitivity to TRAIL induced apoptosis, as shown in Figure 7.

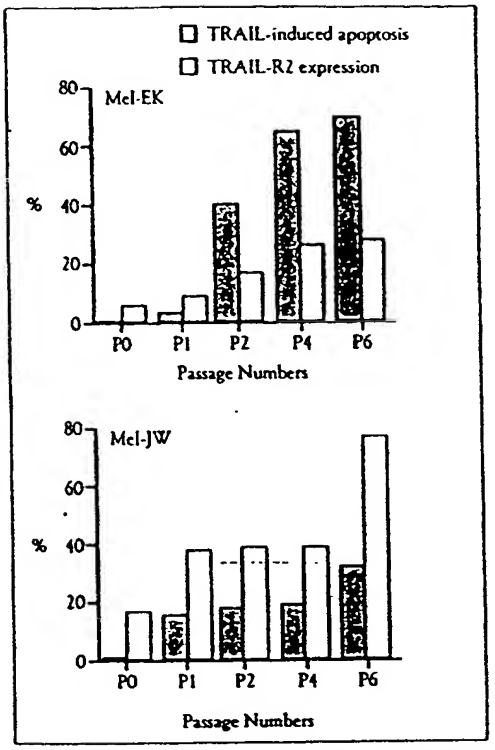

Figure 7. Sensitivity to TRAIL-induced apoptosis and TRAIL-R2 expression on fresh isolates and subsequent passages of cultures established from the fresh biopsies

Discussion

The findings described above, showing that CD4 T cells appear to kill melanoma cells by TRAIL-induced apoptosis rather than by perforin/granzyme dependent mechanisms, provide potentially important insights into mechanisms of tumour control by the host; e.g. it has been known for some time that CD4 T cells appear to predominate in regressing primary melanoma (5). It seems possible that this is due to apoptosis of the tumour cells due to TRAIL expressed on the surface of the CD4 T cells. CD8 T cells also express TRAIL but do not predominate in early regressing melanoma. The reasons for this are not clear but may be related to a more rapid turnover and shorter half-life compared to CD4 T cells. Studies on two animal models have also shown that receptor-induced apoptosis of tumour cells appears important in tumour surveillance and rejection, and provides strong support for the importance of ligands such as TRAIL (and possibly FasL and TNF-α) in tumour rejection (10, 11).

A second important insight from the studies on CD4 T cell-mediated killing of melanoma was the observation that melanoma cells that were resistant to TRAIL were also resistant to killing by CD4 T cells (12). This result implies that studies on factors which are responsible for resistance to TRAIL may also increase the effectiveness of immunotherapy by combining it with reagents that overcome resistance to TRAIL. Our studies suggest that inhibitors of NF-κB activation may be one approach to reducing resistance to TRAIL induced apoptosis. Some chemotherapy reagents, such as Temozolomide, may have this action (19) and newly-described proteasome inhibitors, such as PS341 (20), may also prevent activation of NF-κB. A number of chemotherapy agents were shown to sensitise cancer cells to TRAIL (21, 22) and may provide a basis for combination with immunotherapy.

Studies on blood lymphocytes have shown that TRAIL expression is not confined to the CD4 T cell population but may also be detected on CD8 T cells, monocytes and NK cells. Levels of TRAIL on these cells are barely detectable in normal subjects but are present on lymphocytes from most patients with melanoma, even for long periods after removal of primary melanoma and in the absence of recurrent disease. This may indicate that there is a source of melanoma antigen perhaps in regional lymph nodes that activates a small proportion of lymphocytes in the circulation, particularly the CD4 subset. Exposure to IFN-α2 induces a dramatic increase in TRAIL expression, particularly on the CD4 subset. This was mostly evident in blood lymphocytes from patients with a history of primary melanoma (stage I and II) and regional lymph-node disease (stage III). These findings would be consistent with previous reports that TRAIL expression was increased to higher levels on activated lymphocytes by IFN-α (13). In contrast to IFN-α2, IFN-γ was more effective in increasing TRAIL expression on CD8 T cells and monocytes.

Although TRAIL was expressed on CD8 T cells and NK cells, its role in killing by these cell types appears to be of minor significance, presumably because they kill normally by the granule exocytosis mechanism. When the latter is blocked by depletion of calcium, TRAIL was shown to mediate some of the killing by LAK cells but other TNF ligands may also be involved.

Blood lymphocytes from patients with disseminated melanoma had a relatively low increase in TRAIL expression in response to IFN-α compared to normal subjects and patients with stage I, II and III melanoma. This was mainly evident in the CD4 rather than the CD8 T cell subset and no differences were seen in TRAIL expression on monocytes. The reasons for this are not clear. "Resting" PBL from the stage IV patients expressed TRAIL at similar levels to those in other patients so it was not due to a lack of antigenic stimulation. It is possible that particular subsets of CD4 lymphocytes expressing TRAIL in response to IFN-α2 are depleted in these patients or that tumour-related factors in their circulation inhibit the IFN-α2 induced response. Support for the latter explanation came from studies on supernatants collected from melanoma cell lines. Supernatants from cell lines that were immunosuppressive in anti-CD3 lymphocyte proliferative assays were shown to completely inhibit upregulation of TRAIL by IFN-α2. The mechanisms involved and the nature of the suppressive factor are as yet unknown. Should tumour-derived factors be involved in the suppression of IFN-α2 upregulation of TRAIL, it would provide a further explanation for variation in responses to IFN-α2.

These results, showing that IFN-α2 upregulates TRAIL expression on circulating T cells, raise a question as to whether IFN-α2 may mediate its anti-tumour effects *via* TRAIL expression. Support for this hypothesis comes from studies showing that responses to IFN-α2 correlated with CD4 T cell infiltrates into the melanoma (7). IFN-α2 does however have many other effects and further study is needed to examine whether TRAIL may be an important mediator of IFN-α2 and even IL-2 mediated anti-tumour effects.

This view of the importance of TRAIL has important implications for therapy for two reasons. One is that treatment may be made more effective by optimisation of protocols to achieve high TRAIL expression on CD4 T cells. Secondly, measures that may increase the sensitivity of melanoma to TRAIL-induced apoptosis may be important in achieving clinical responses. Our past studies have shown that the sensitivity of melanoma cells to TRAIL is determined largely by the level of expression of the death receptors, particularly TRAIL-R2 (17). In addition, activation of NF-κB appears to be involved and to be superimposed on the level of apoptosis determined by the level of TRAIL-R2 expression. TRAIL appears to mediate apoptosis of melanoma *via* mitochondrial pathways (23) and inhibitors of this pathway, such as Bcl-2 and IAP 1 and 2 and XIAP, may be important in the regulation of TRAIL-mediated responses. In view of the low TRAIL death receptor expression in fresh melanoma tissue, regulation of the level of the death receptors may also be an important strategy in improving responses to therapy. The death receptor TRAIL-R2 is upregulated by p53 (24) and consequently chemotherapy and other DNA damaging agents (25) may reverse resistance to TRAIL by upregulation of death receptors.

These optimistic studies on TRAIL need to be tempered by studies showing possible toxicity to human liver cells (26) and our findings that freshly-isolated melanoma cells appear less sensitive to TRAIL than cultured cells (27). Nevertheless, the present studies provide new insights into the possible mode of action of vaccine and cytokine therapy and provide a framework for future studies that focuses on the nature and susceptibility of tumour cells and which monitors the level of TRAIL expression on key effector cells.

Conclusions

TRAIL is a member of the TNF family that induces apoptosis in approximately two-thirds of cultured melanoma. CD4 T cells appear to kill melanoma by expression of TRAIL on their surface. Melanoma cells that were resistant to TRAIL were also resistant to killing by CD4 T cells. IFN-α, and to a lesser extent IFN-γ, appears to have an important role in upregulation of TRAIL on the surface

of CD4 T cells. IFN induced expression of TRAIL is also influenced by factors released from melanoma cells and this may account for the relatively low expression of TRAIL on CD4 T cells from patients with disseminated melanoma. These findings raise a question as to whether the therapeutic effects of IFN-α2 may be *via* expression of TRAIL on T cells and if this were the case it would be expected that responders would have high TRAIL expression on their T cells and TRAIL-sensitive melanoma cells. These insights may also provide a way forward to improve results from treatment with IFN (and IL-2) by combining them with agents that sensitise melanoma cells to TRAIL-induced apoptosis.

References*

1. McGovern VJ. Spontaneous regression of malignant melanoma. In: Blaustein A, ed.; *Melanoma: Histological Diagnosis and Prognosis*, New York: 1982; 138-147.
2. Clemente CG et al. Prognostic value of tumor infiltrating lymphocytes in the vertical growth phase of primary cutaneous melanoma. *Cancer* 1996; 77: 1303-1310.
3. Hersey P et al. Current research on immunopathology of melanoma: analysis of lymphocyte populations in relation to antigen expression and histological features of melanoma. *Pathology* 1985; 17: 385-391.
4. Brocker EB et al. Inflammatory cell infiltrates in human melanoma at different stages of tumor progression. *Int. J. Cancer* 1988; 41: 562-567.
5. Tefany FJ et al. Immunocytochemical analysis of the cellular infiltrate in primary regressing and non-regressing malignant melanoma. *J. Invest. Dermatol.* 1991; 97: 197-202.
6. Hakansson A et al. Effect of IFN-alpha on tumor-infiltrating mononuclear cells and regressive changes in metastatic malignant melanoma. *J. Interferon Cytokine Res.* 1998; 18: 33-39.
7. Stalder T et al. Fas antigen is the major target molecule for CD4+ T cell-mediated cytotoxicity. *J. Immunol.* 1994; 152: 1127-1140.
8. Trapani JA et al. CTL granules: evolution of vesicles essential for combating virus infections. *Immunol. Today* 1999; 20: 351-356.
9. van den Broek ME et al. Decreased tumor surveillance in perforin-deficient mice. *J. Exp. Med.* 1996; 184: 1781-1790.
10. Medema JP et al. Immune escape of tumors *in vivo* by expression of cellular FLICE-inhibitory protein. *J. Exp. Med.* 1999; 190: 1033-1038.
11. Djerbi M et al. The inhibitor of death receptor signaling, FLICE-inhibitory protein defines a new class of tumor progression factors. *J. Exp. Med.* 1999; 190: 1025-1031.
12. Thomas WD and Hersey P. TNF-related apoptosis-inducing ligand (TRAIL) induces apoptosis in Fas Ligand-resistant melanoma cells and mediates CD4 T cell killing of target cells. *J. Immunol.* 1998; 161: 2195-2200.
13. Kayagaki N et al. Type I interferons (IFNs) regulate tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) expression on human T cells: A novel mechanism for the antitumor effects of type I IFNs. *J. Exp. Med.* 1999; 189: 1451-1460.
14. Griffith TS et al. Monocyte-mediated tumoricidal activity *via* the tumor necrosis factor-related cytokine, TRAIL. *J. Exp. Med.* 1999; 189: 1343-1353.
15. Zamai L et al. Natural killer (NK) cell-mediated cytotoxicity: differential use of TRAIL and Fas ligand by immature and mature primary human NK cells. *J. Exp. Med.* 1998; 188: 2375-2380.
16. Johnsen AC et al. Regulation of APO-2 ligand/TRAIL expression in NK cells-involvement in NK cell-mediated cytotoxicity. *Cytokine* 1999; 11: 664-672.
17. Zhang XD et al. Relation of TNF-related apoptosis-inducing ligand (TRAIL) receptor and FLICE-inhibitory protein expression to TRAIL-induced apoptosis of melanoma. *Cancer Res.* 1999; 59: 2747-2753.
18. Zhang XD et al. Differential localization and regulation of death and decoy receptors for TNF-related apoptosis-inducing ligand (TRAIL) in human melanoma cells. *J. Immunol.* 2000; 164: 3961-3970.
19. Thomas WD et al. Temozolomide inhibits activation of NF-κB and sensitizes melanoma cells to TRAIL induced apoptosis. *Proc. Am. Assoc. Cancer Res.* 2000; 41: A451.
20. Teicher BA et al. The proteasome inhibitor PS-341 in cancer therapy. *Clin. Cancer Res.* 1999; 5: 2638-2645.
21. Gliniak BC et al. Enhanced therapeutic efficacy of TRAIL/APO2L in combination with CPT-11 in the treatment of human tumor xenografts. *Proc. Am. Assoc. Cancer Res.* 2000; 41: A450.
22. Mizutani Y et al. Synergistic cytotoxicity and apoptosis by TRAIL and adriamycin against bladder cancer cells. *Proc. Am. Assoc. Cancer Res.* 2000; 41: A449.
23. Thomas WD et al. TRAIL induced apoptosis of melanoma is associated with changes in mitochondrial membrane potential and perinuclear clustering of mitochondria. *J. Immunol.* 2000; In press.
24. Sheikh MS et al. p53-dependent and -inde-

*The reference numbers written in bold-type indicate the selected abstracts which follow this article

pendent regulation of the death receptor KILLER/DR5 gene expression in response to genotoxic stress and tumor necrosis factor alpha. *Cancer Res.* 1998; 58: 1593-1598.
25. Chinnaiyan AM et al. Combined effect of tumor necrosis factor-related apoptosis-inducing ligand and ionizing radiation in breast cancer therapy. *Proc. Natl. Acad. Sci. USA* 2000; 97: 1754-1759.
26. Jo M et al. Apoptosis induced in normal human hepatocytes by tumor necrosis factor-related apoptosis-inducing ligand. *Nat. Med.* 2000; 6: 564-567.
27. Nguyen T et al. Relative resistance of fresh isolates of melanoma to TRAIL induced apoptosis. *Clin. Cancer Res.* 2000.

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